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M.T. Townsend.

Some Methods Of Studying The Metabolism Of Hibernating Animals

SOME METHODS OF STUDYING THE METABOLISM OF HIBERNATING ANIMALS

BY

MYRON THOMAS TOWNSEND
B. S., Bates College
1918

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN ZOOLOGY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1921

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UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

JUNE 4 1921

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY MYRON THOMAS TOWNSEND

ENTITLED SOME METHODS OF STUDYING THE METABOLISM OF HIBERNATING ANIMALS

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

Victor E. Shelford
In Charge of Thesis

Henry Sharp
Head of Department

Recommendation concurred in*

Committee on Final Examination*

*Required for doctor's degree but not for master's

47663

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I Introduction

Hibernation has been studied by various workers,and much attention has been given to hibernating mammals in the hope of finding some internal physiological explanation for the phenomenon. More recently emphasis has been laid on the condition as observed in insect pests and its bearing upon methods for their control. A great many phases of the subject have not yet been studied,and especially is this true of certain ecological relations to external environment as opposed to the internal physiological side of the problem. It is my purpose to study the phenomenon of hibernation from an ecological point of view with the hope of throwing new light on some of the economic or purely scientific problems involved,especially as regards this condition in cold blooded animals.

With this in view it seemed best at the outset to choose an animal for experimentation large enough so that various determinations such as body temperature,blood analyses,etc. could be made if desirable.The common toad,Bufo americanus Le Conte,seemed to fit these requirements very well,being a land animal during the greater part of its life-history,cold blooded, accustomed to pass the winter in a state of hibernation,and large

enough to admit of easy determinations of body temperatures, blood analyses, etc. Accordingly, the first part of the paper is given over to a study of various methods of keeping toads in captivity for experimental purposes. In the course of this study, the importance of the water content of the animals became evident (Part 3) and led to some preliminary investigation as to the regulatory mechanism involved, the results of which are discussed in Part 4. The remainder of the paper is occupied with descriptions of apparatus for studying the carbon dioxide output of the animals together with some preliminary experiments with these. The data so far gathered is not sufficient to warrant the drawing of any new conclusions regarding the metabolism of hibernating animals, but such data will be gathered later when the apparatus and methods here described have been used to a greater extent.

II. Materials

In the fall of 1919, I began my work on toads by attempting to collect specimens in the vicinity of Urbana, Ill. At that season of the year I found them to be very scarce, and after two or three weeks of persistent searching I had procured only two or three toads and a few snakes. Later observations indicated that the toads must have been in the vicinity, but at that season they were widely scattered and well hidden so that there were no definite places where they could be collected in

numbers. Accordingly a dozen Florida toads were procured from the Chicago Biological Supply House. They were shipped in a screen-sided wooden box half full of oak leaves, presumably from their native habitat.

The toads and leaves were transferred to a wire screen cage (14 in. by 10 in. by 8 in.) and placed in the Vivarium green-house. The cage was placed on strips of wood above a water table and a continuous stream of water was kept flowing under it to keep the air moist. The temperature of the room was about 68° F. About the middle of December, upon examining the cage, I found one toad dead, and I then observed that while the water ran under the cage continually, the oak leaves immediately surrounding the toads were quite dry and brittle. I lowered one end of the cage into the water and about an hour later found all the toads congregated in that end and several of them swelled up to a considerable size because of the amount of water which they had imbibed. This experience served to emphasize the importance of a readily available water supply for keeping toads in a healthful condition, and to meet this need better, I removed the toads to a new location. I returned the animals to the screen-sided box and placed this, screen side down, on top of a galvanized iron tank of water. The rest of the tank was covered by a board. By this means I hoped to obtain a sufficient degree of saturation of the air in the box to keep the toads in a healthful condition. The whole enclosure was placed on a brine coil in a cold room (temperature about 11° C.) in

order to lower the rate of metabolism of the animals and keep them hibernating if possible. In this inclosure the toads remained apparently dormant, but on Jan. 5, 1920, I found that two more had died and from this time on they slowly died off until at about the middle of the month all were dead. The leaves in the cage at this time were observed to be quite dry which would seem to indicate that the air in the box was not saturated with moisture as much as I had hoped it would be.

Some field observations during the spring of 1920 further emphasized the importance of moisture in the environment of the toad. At this time I began to collect animals from the vicinity of the lily pond on the south campus. Here the toads gather each spring to breed and lay their eggs in the pond. One or two cold nights occurred late in the spring and I found that at such times the toads sought shelter in various places, and quite a number of them fell into the concrete pits in front of the cellar windows of the green-houses near by. These pits are about three feet deep and four feet wide and covered by open iron gratings thru which the toads easily fell. The windows into the cellar were kept closed and the toads were thus easily trapped. The bottoms of these pits were usually found covered with a layer of leaves and soil in which the animals burrowed, and it was observed that when the leaves and soil dried up, the toads buried in them were also found to be dried up and dead. If the leaves and soil in a window pit were found damp usually from one to eight or ten healthy toads could be found by over-

turning the leaf mulch. In this way about 50 specimens were collected and brought to the vivarium, where several methods of keeping them alive were tried, as follows -

Three toads were placed in a screen cage (12 in. by 12 in. by 12 in.) and this was placed on the surface of the ground in a sheltered corner on the north side of the vivarium. No sunlight ever reaches the spot and the soil is always moist. A shallow depression was dug in the soil and the cage was placed in this and banked up about two inches on all sides with moist earth. A few leaves were placed in the cage. Although the bottom screen of the inclosure was all that intervened between the toads and the moist surface of the earth, and the air in the inclosure might be expected to be reasonably moist and cool, nevertheless a few days later the animals were found to be stretched out on the cage floor, too stiff to allow ready movement of the limbs. They were removed to the laboratory and held under a cold water faucet and two of them responded by a very slight movement. Repeated drenching with water completely revived these two and they were apparently as healthy as before their confinement. The third toad did not recover. Apparently the moisture content of his body had been reduced below the minimum. The few leaves contained in the cage were found to be partially dried up when the animals were removed.

The foregoing observations would seem to indicate that the toad must be able to come into direct contact with moist soil, perhaps to burrow in it in order to maintain the proper

moisture content necessary for health. This conclusion is further borne out by experiments with another type of cage as follows:

Several flowerpots were prepared for use as inclosures; (See Plate I, Fig. 1); each was filled 2/3 full of soil, provided with a wire screen cover and set in a saucer of water. The soil in the pot soon absorbed a large amount of water and became of the consistency of wet mud. One or two toads were placed in each pot and the covers weighted down to prevent their escape. In this type of cage so far as I have tried it, the toads live in perfect health. Usually the animal burrows backward into the mud and sometimes completely buries itself out of sight by this means. When dug up the toads were always fat and plump and wide awake. Apparently their surroundings of wet mud furnished just the right kind of environment for them.

A number of toads were kept in a large aquarium tank with about two inches of water in the bottom and provided with scattered bricks and boards for them to climb onto. Here they remained apparently in good health. Several occupied a pile of wet soil in one end of the tank, burrowing deep in the mud, frequently in pairs, the male clasping the female.

As a means of keeping the toads thru the summer, I arranged an inclosure in an open screen-covered room connected with the green-house. A corner of the room underneath some tables was fenced off with wire screen two feet high. A pile of black dirt was dumped on the concrete floor in the middle of the

cage and this was partially covered by a large flat box turned upside down to furnish a shady retreat. A stream of water was kept flowing over the floor and partially dammed to furnish a small artificial pond. In this inclosure forty toads were left during the summer . As a means of feeding them, I suspended an electric light above the cage and this was turned on every evening during the summer to attract insects. A supply of angleworms was also dumped on the pile of dirt. In this inclosure and without further care the majority of the toads lived thru the summer. In the latter part of September, thirty out of the original forty were still alive and active, altho several, especially the males,were very thin. Efforts were made to feed them with insects caught in the fields with an insect net, and a special feeding device was arranged for this purpose, as shown in Fig. 1, Plate 1. The insects were released from the collecting jars inside the screen cage C, the meshes of which were very small. Then six or eight toads were placed in the cage and allowed to feed. As the insects were usually brought in late in the afternoon, the light L was hung near enough to aid the animals in catching their prey during the evening. The toads were allowed to come and go at will from the cage thru the dark passage A B. This passage being kept dark, very few of the insects escaped thru it, most of them being attracted to the other end of the cage by the light. Two or three mason jars full of insects were usually placed in the cage and opened at once, and the insects crawling or flying out of the jars furnished a continual

supply of food for the toads for some time. Such a feeding device seemed to be very successful, but the supply of insects obtainable at that season proved to be inadequate for the thirty animals and not many of them were in good condition when cold weather set in. All died during the early winter. Death may have been due to the cold weather rather than to the lack of feeding. The toads had crawled under the box in the center of the cage and burrowed a little in the shallow earth there, but this furnished only a scanty protection against the severe cold. Furthermore, the freezing of the soil rendered the moisture in it unavailable to the animals and this may have been a factor. Experiments described earlier in the paper have indicated how important a factor in the toad's environment is contact with moist soil at least during the early summer. Presumably the same principle holds good for the animal during hibernation, and a condition of physiological drought brought about by cold weather and lack of protection may be fatal.

III. The Water Content of the Toad

During the spring of 1921 some experiments were performed with a view to determining the minimum water content necessary to the life of the toad. A number of males were collected from the breeding ponds and placed in cages in the laboratory without water. They were weighed at intervals and the loss in weight recorded until a few of them had completely dried up

and died. This was first observed at the end of 52 hours. Then all were transferred to aquaria containing water to a depth of about an inch and allowed to absorb all the water they would. Several which had become quite dry and were beginning to stiffen, completely revived under this treatment and absorbed a great deal of water. The changes in weight were recorded and percentages of water lost and gained at various points in the experiment calculated. The results are summarized in Table I. It will be noted that the average percentage of water lost by those which were not killed by the drying process was 36.8%, while that of the animals which did not survive was 46.5%. Those which revived when given access to the water increased their weight by 48.9 gms. on an average, practically doubling the average weight of the survivors at the end of the drying process. (49.9 gms.) The average weight of the dead animals was 36.1 gms.

The above observations and experiments have emphasized the importance of readily available moisture in the environment of the toad, and while the experimental data is limited, it nevertheless seems to indicate that a toad can carry a relatively enormous amount of water in its body. Perhaps this capacity for retaining moisture may be a factor in enabling the toad to live on land away from the immediate vicinity of the ponds. We have seen that in a comparatively dry atmosphere such as would obtain in the laboratory the animals lose moisture and may die in the comparatively short period of 52 hours. (In one experiment five toads died in 41 hours). This fact may perhaps be correlated with the

reactions of the animal to gradients of evaporating power of the air as shown by Shelford (1914) and indicates that these reactions tend to keep the animals in their optimum environment in moist situations.

The question as to where in the toad's body the water is taken in and where it is retained has not been answered satisfactorily. I have never seen a toad drink in the ordinary sense of the term and it seems unlikely that any appreciable amount of liquid is taken in in this way. When a toad which has absorbed a large amount of water is handled roughly for a few minutes, much of the water is expelled thru the anus and possibly this might be where the water is taken in. A more likely place, however, would seem to be thru the skin by some sort of osmotic action. Possibly it is stored in the lympth spaces beneath the skin and passes from here thru the wall of the bladder, when the animal is disturbed, or it may be stored directly in the bladder. These points have not yet been studied. Some preliminary work has been done on the skin.

IV. The Absorptive Force of the Toad's Skin

Reid in 1890 studied the osmotic action of the skin of the frog and reached the following conclusions:

1. The normal direction of easiest osmotic transference of fluid thru the living skin of the frog is in the direction from the outer toward the inner surface.

2. The transfer of fluid in the above direction is intimately associated with the condition of the tissues. Conditions or agents tending to depress vitality diminish the transfer in the normal direction whereas stimulants tend to augment it.

Reid suggests the possibility of a definite absorptive force exercised in the living skin which might explain some of his results and he records three experiments in which his discharging osmometers showed a definite action taking place thru the skin when the same solution was placed on both sides of the skin, thus eliminating the possibility of osmotic action in the ordinary sense of the term.

It seemed worth while to determine whether these same principles applied to the skin of the toad with its more terrestrial mode of life than that of the frog. For this purpose, I devised a simple type of osmometer shown in Fig. 3, Plate 1. An ordinary Harvard staining jar A, is provided with a two-hole rubber stopper B, in which are inserted two glass tubes, C and D. The lower end of tube C is flush with the lower side of the stopper, while tube D extends about ½ inch below. To prepare the apparatus for use, stopper and tubes are removed and a small piece of skin,E, is placed over the longer tube (the inside surface of the skin being placed next to the glass for example). The skin is drawn tight and sealed in place with sealing wax F. The jar is now filled level full with the solution for the experiment and the stopper is inserted in place. This forces some of the liquid up into tube C, and if the apparatus is properly

made, no air bubbles will remain in the jar. The same liquid or one of different density is now poured into the top of tube D to a convenient height and the levels of the two columns of liquid adjusted until they are the same. The apparatus is now ready for use. It is set aside and readings are taken every few hours by measuring and recording the heights of the two columns of liquid. If osmotic action takes place thru the skin from the outside to the inside, the liquid in tube D will rise and that in tube C will fall; if from the inside of the skin toward the outer surface the level of liquid in tube C will rise and that in D will fall. Thus we have a means of measuring both the direction and the speed of any osmotic or other action which may take place.

The tubes used in these experiments had an inside bore of 7 mm. and were of uniform size. Each tube was flamed before using to remove any trace of grease which might affect the capillary action. Tubes having an inside bore of about 7 mm. were found to be most convenient for several reasons. If smaller ones were used, it was found to be difficult to pour the liquid down into them in adjusting the level. With a tube as large as 7 mm. in diameter, a pipette may be thrust down into it and the level of the liquid adjusted by means of this. On the other hand, if a large-ended thistle tube is used, the piece of skin becomes limp and stretches and sags in such a way as to make the results inaccurate.

Results with this type of osmometer were recorded on the experiment sheets as shown in Table II. Two columns were arranged for each osmometer, one marked (Column 4) referring to the tube which was closed by the piece of skin, the other marked - (Column 5) for the open tube. The heights in millimeters of the two columns of liquid were recorded at intervals in their respective columns. The difference between the two columns between each two successive readings was then computed (Column 6) and the number of hours elapsed is calculated (Column 3). Results may be recorded in tabular form, showing the maximum difference between the two columns during the course of the experiment, as in Table III. This, however, does not show the rate of action taking place at various times during the experiment. This may be shown by plotting the results on coordinate paper, representing on the ordinate the number of millimeters by which the height of the positive column exceeds that of the negative column of liquid and on the abscissa the time in hours. Graphs I to IX illustrate this method of recording results. Table III and Graphs I to IX give a summary of the experiments which have been performed to date.

In the experiment represented by Graph I, the main reservoir of the osmometer and the negative tube were filled with distilled water, and the positive tube was filled with a solution of higher osmotic pressure (5% cane sugar in distilled water). Two osmometers were used, one represented by curve x on the graph, being arranged with the skin placed in the apparatus with the

outer surface down; the other represented by curve y arranged with the inside surface of the skin down, thus placing the liquid of greatest density on opposite sides of the membrane in the two parts of the experiment. In other words curve x represents results with the cane sugar solution of the inside surface of the skin, curve y with the sugar solution on the outside surface of the skin. A comparison of the two curves shows immediately that the osmotic action took place readily when the sugar solution was on the inside of the skin but not at all when the solution of greatest density was on the outside of the skin until late in the experiment, when the action ceased in curve x and began in curve y. If we assume that the tissues of the skin had died at this point, then the experiment would seem to corroborate Reid's conclusion regarding the frog's skin, namely that the normal direction of easiest oxmotic flow in the living skin is from the outside toward the inside.

Graph II illustrates a similar experiment in which the fluid of greater density was some water expelled from the bladder by toads when handled roughly as above described. Presumably this liquid was of a greater density than distilled water, altho this has not been proven. The general trend of the curves is similar to that of Graph I, but since nothing is known of the nature of the liquid used, no definite conclusions can be drawn from the experiment at the present time.

A number of experiments were performed using the same liquid on both sides of the membrane. Three liquids were used

as follows:

1. 0.6% Nacl (Approximately isotonic).
2. 5.0% alcohol (as a stimulant).
3. A weak solution of chloroform in distilled water (as a depressant).

In the earlier experiments of this kind, the skin was removed from various parts of the animal, sometimes from the back, sometimes from the belly or from the side or the breast. Results seemed to indicate that a decided flow of liquid took place thru the skin from the outside to the inside and that the strength of this action varied with the solutions used and with the condition of the animal at the time when the skin was removed. Later experiments in which the skin was always removed from the belly side gave different results, showing practically no action except in the case of the chloroform solution. These experiments are summarized in Table III, Nos. 2 - 6. A further study of the earlier data suggested that the absorptive force might vary according to the source of the skin on the animal's body. To test this point, Experiment 8 was set up, using skin taken from the back of the animal. The results proved to be quite different, as is evident when Graphs III and IV are compared. In these two experiments, conditions were exactly the same so far as known, except that the skin used in Graph IV was taken from the back of the animal, while that in III was taken from the belly side. Apparently quite a difference exists in the absorptive force of skin taken from these two regions

of the body, the action being strongest thru the back skin. This fact is also shown by later work. Possibly this difference may be correlated with differences in the structure of the skin, the presence of poison glands on the dorsal surface or some other factor not yet studied.

Graphs V, VI, and VII show the results of an earlier experiment in which the skins of three toads were compared as follows: Skin No. V was taken from a normal toad. No. VII was taken from a toad which had been allowed to dry up and die, and skin III was taken from a partially dried specimen, representing roughly an intermediate stage between the other two. Results indicated that the absorptive force varied markedly with the condition of the animal when killed. Unfortunately however, the skin for these experiments was not taken uniformly from the same part of the body, that for the alcohol and chloroform being derived from the back and that for the Nacl from the side. Hence, in the light of the later work indicating that the skin from different sources differs in its absorptive force, this experiment needs careful corroboration.

This was begun in experiment 9, Table III, in which a partially dried toad was used, one which had lost 31 gms. in weight. Skin was removed from the belly side and from the back and the two compared. Results were quite striking as shown by Graphs VIII and IX, chloroform accelerating the action when skin from the belly was used, whereas alcohol produced a strikingly similar result in the case of the back skin. What the signifi-

cance of this may be, cannot be stated at the present time, but in this connection it will be noted that in experiments 2 to 6, (Table III), where skin from the belly was used, chloroform was the only solution which showed any noteworthy results.

As regards skin from a normal toad in contrast to that of one partially dried, Graphs IV and IX may be compared and show striking differences in the results in the different solutions.

Conclusions

The data so far gathered cannot be considered as sufficient to warrant the drawing of any definite conclusions. It is merely suggestive. As mentioned above, Reid (1890) as a result of his work on the frog's skin, suggested the possibility that some positive absorptive function might be exercised by the living cells of the skin which would explain his results. In other words, he put forward the hypothesis that the living skin exercised some force comparable to a force pump and that this force ceased with the death of the tissues. This seems so striking an assumption that very careful work must be done and a great deal of data gathered before it can be considered as proven. I merely wish to call attention at this point to certain aspects of my experiments so far that seem to fit into his theory. In Graph I as before mentioned, the osmotic action seems to go readily from the outside surface of the skin to the inside, but

not at all from the inside surface toward the outer one until late in the experiment. At approximately the point where the curve x ceases to rise, the curve y begins to rise. This would be quite in keeping with a theory that the tissues had died at this point and the absorptive force exerted by them had then ceased. Hence curve x ceased to rise. The skin represented by curve y also having died at about the same time now acted as any non-living membrane and osmotic action took place toward the liquid of greatest density. Why curve x should fall to nearly zero is not so readily explainable, but bubbles in the liquid confused the readings in this part of the experiment and hence perhaps the curve should not have gone so low. No bubbles formed in the other half of the experiment to confuse results.

Another point worthy of note is illustrated in Table II. At the bottom of the table is a transverse column headed "increase," derived as follows. If we glance down Column 4, for example, we note that this column actually rose only one millimeter in height, and a number of other columns did not rise at all. In the chloroform part of the experiment, the positive column rose 2.5 mm. The difference in height of the two columns was due then in a number of cases to a lowering of the column in the negative tube, not to a rise in the positive side. At first thought this would seem to indicate that no "absorptive force" had operated, that the difference between the two columns was due perhaps to evaporation in the negative tube. The slight rise in the positive column might have been due to temperature

which unfortunately was not well controlled. But an examination of other experimental sheets in the series shows that in a number of cases a definite rise in the positive column took place. These observations are summarized in Table V. It will be noted that in Experiment 9 A, the chloroform solution produced a definite rise of 6.5 mm. This could hardly be due to temperature although this point needs to be tested carefully. If now the results are due to evaporation in the negative tube, then the question arises as to why evaporation did not lower the liquid in the positive tube equally. The two tubes unfortunately were not always of equal height, the greatest difference between them in any osmometer being 32 mm. In most cases they were nearly equal in height and in several cases the shortest tube was the one in which the lowering of the liquid did not take place. It seems very unlikely then that unequal evaporation was responsible for the results obtained. If now we return to our working hypothesis that the skin exercises a definite absorptive function, this phenomenon might be explained thus:

Evaporation takes place equally in both tubes, but as fast as the liquid evaporates in the positive tube, it is replaced by liquid drawn in by the absorptive force of the skin, and in some cases this liquid drawn in exceeds the amount lost by evaporation and the level of the column of liquid rises. Thus the column in the negative side would be lowered by evaporation on that side plus the liquid removed to the positive tube by the absorptive force of the skin.

More accurate methods must be used and more experimental data must be gathered before we may consider any of these points as proven, but the field seems to be a promising one and worthy of more careful investigation.

V. Respiration Apparatus

As a convenient index to the rate of metabolism of the animals experimented upon, I have chosen to use the rate of CO_2 production, and with this in view, a study was made of the Biometer described by Tashiro ('17) and of the titration apparatus described by E. J. Lund ('19). These were modified and enlarged in various ways to suit the needs of the present study. A description of these apparatuses as modified follows.

Apparatus for Obtaining CO_2 - Free Air

An essential part of any apparatus for the determination of CO_2 is a means of thoroughly cleaning the various parts of it. This involves not only the use of cleaning fluids but also the use of dry air, free from CO_2, with which the various chambers and tubes should be rinsed between each two readings. Figure 4, Plate 1, shows a diagram of such an apparatus, modeled after that described by Tashiro.

When a supply of pure air is to be prepared bottle H is full of a 20% solution of NaOH, and bottle F is empty. The suction pump attached to tube A is started, screwcocks D and O

are closed and C and E opened, so that the suction from the pump exhausts the air in bottle B and tube R and creating a partial vacuum in the bottle F, thus drawing the NaOH from bottle H thru the tube G and into the bottle F. Bottle H is thus emptied and is in turn filled with air drawn in thru tube I (screwcock N being open and K closed). This air has been sucked thru the series of alkaline towers J, in which process it is bubbled thru the solutions of NaOH contained in them and thus freed from any CO_2 it might contain. Thus as the suction continues, bottle F is gradually filled with NaOH and bottle H is filled with CO_2-free air which is now available as a supply for cleaning the Biometer. When bottle H is almost emptied of its contents of NaOH the suction pump is stopped, screwcocks E and N are closed tightly and the supply of air is ready for use. Part of the NaOH from bottle F siphons over into H but this soon stops if everything is tight. Usually this air is forced under pressure thru the Biometer and the method of precedure is as follows.

Screw-cocks D and E are opened to allow the air to enter bottle F and then screwcock K (previously closed tight) is opened. The NaOH in bottle F now siphons thru the tube G and down into bottle H. The pressure in bottle H forces the CO_2 - free air contained therein thru the tube P into bottle L where it is bubbled thru weak NaOH again as an added precaution and then passes out thru tube M and into the Biometer. With the bottles F and H of the proper size (10 gallons) a continuous stream of CO_2 - free air may be forced out thru tube M for more

than an hour. The time required to refill the bottle H with pure air is also about an hour.

Titration Apparatus for Determining CO_2

Plate 2 represents an apparatus for the determination of CO_2 by titration, applying the principle used and described by E. J. Lund (1919), namely, the titration of standard HCl against standard $Ba(OH)_2$. The apparatus is used as follows.

Standard $Ba(OH)_2$ from bottle A is admitted thru tube B into the side-arm burette C, and is titrated against standard HCl similarly admitted to the burette D. When the two burettes are filled screw-cocks 1 and 2 are closed. Pinch-cock 3 is now opened and a known amount of $Ba(OH)_2$ is admitted into the leveling bulb E. This is now diluted with distilled water admitted thru tube F and the apparatus is ready for use. Tube G is connected to the respiratory chamber (to be described later) in which the animal is contained, and tube H leads thru the drying tube I to the suction pump. By means of this suction pump on the one side and the pure air being admitted under pressure to the respiratory chamber on the other side, as will be described later, a current of air is kept passing thru the tube G and bubbling up thru the solution in the bulb E. The CO_2 contained in the air unites with some of the $Ba(OH)_2$ to form an insoluble precipitate of Ba_2CO_3 and the excess $Ba(OH)_2$ can be can be titrated with standard HCl. After the experiment has

been continued for an hour or any other period, the suction pump is stopped, screw-cock No. 5 is closed quickly so as to catch all of the solution in E (this can be done if tube G is of rather small size) and the solution is ready to titrate. A few drops of phenolphthalein indicator are admitted to the bulb from the thistle tube K and the solution is titrated with standard HCl from burette D. The HCl used represents the excess $Ba(OH)_2$, i.e. that which has not united with CO_2 to form Ba_2CO_3. By subtraction the amount of $Ba(OH)_2$ used is determined and from this figure the amount of CO_2 which has united to form Ba_2CO_3 is calculated. This represents the amount of CO_2 in the air breathed out by the animal in the chamber during the experimental period.

The Respiratory Chamber

The respiratory chamber for use with the apparatus is shown in Fig. 6, Plate 2. An ordinary bell-jar L with the tubulature (at M) near its bottom, is inverted inside a battery-jar N N and covered by an ordinary desiccator cover O O. Application of vaseline around the edge of the desiccator cover and the addition of a weight of some size on top will usually make an air-tight connection. A wire cage P P containing the animal experimented upon is placed inside the respiratory chamber. The battery jar N N outside the chamber is kept filled with water which may be of any desired temperature and may be siphoned in from a reservoir above. Thus the temperature in the respiratory chamber

may be kept constant or may be gradually changed according to the needs of the experiment. The chamber is used as follows.

The wire cage P P containing the animal is placed inside the respiratory chamber and the cover O O is vaselined and placed tightly in position, being held down by a weight if necessary. CO_2 - free air under pressure is admitted thru the tube Q, passes down into the water jacket and around thru the glass coil R R, thus being cooled or warmed to the same temperature as the air in the chamber and finally enters the chamber thru the inlet at M. Here it circulates around the cage PP and passes out thru the tube T at the bottom, and thence thru the tube G into the titration apparatus. Since CO_2 is heavier than air, practically all of the CO_2 given off by the animal will sink to the bottom of the chamber and pass out into the titration bulb without difficulty. For certain experiments it might be desirable to regulate the pressure inside the respiratory chamber, in order to avoid subjecting the animal to either a higher or a lower pressure than that of the atmosphere outside. This may be done by regulating the screw-cock controlling the inlet of pure air on the one side and the suction pump on the other side of the apparatus, and as an indicator of the pressure inside the chamber a manometer may be attached to the tube S. The manometer used consists simply of a long glass tube bent slightly in the middle as shown in Figure 7. The end of the tube A is attached to the tube S of the respiratory chamber. End B is left open. Inside the tube at C is placed about one cc.

of Ellison's draft gage oil. If the pressure inside the respiratory chamber increases, this drop of liquid will be forced over toward B and if the pressure decreases it will be drawn toward A. By adjusting the air inlet and the suction pump, the drop may be kept at the center C and the pressure inside the chamber will remain constant at the pressure of the outside atmosphere. Experience seems to indicate that ordinarily there is very little danger of changing the pressure within the chamber enough to influence the metabolism of the animal, but with the manometer attached, the pressure is easily adjusted and this possible source of error is eliminated.

Experiments with the Titration Apparatus

Table VI gives the results of some preliminary experiments performed with this apparatus. The CO_2 output of several toads is given together with other data and calculations which might be of interest. The results are given merely to illustrate the use of the apparatus. The weight of the animal used should always be recorded, together with the length of time during which the animal was kept in the cage. A record of the number of cc. of $Ba(OH)_2$ which were neutralized, i.e. which united with the CO_2 in the air sample to produce insoluble $BaCO_3$ serves as an index to the relative amount of CO_2 produced per toad. From this and the time, the final calculation, given in column 7 may be made, namely: mg. of CO_2 per kg. of body weight per hour.

In column 8 this figure has been divided by the body weight in each case and the results so far as this table is concerned seem to indicate that the CO_2 production bears no direct relation to the body weight. In column 3 some notes on the general condition of the animal at the time the analysis was made are given. These twelve analyses were made during the fall of 1920 using animals which had survived the summer and were being kept in the outdoor enclosure at the Vivarium. About the middle of November, the last of the toads died and no more experiments could be performed. Numbers 11 and 12 are analyses of the same animal on two successive days when the vitality of the specimen seemed to be waning. The animal died soon after, probably on November 15. A marked decrease in the CO_2 output from this toad is noticeable on the second day. In these two experiments the temperature was controlled by siphoning ice-water into the outer jacket of the respiratory chamber. Outdoor temperatures on these two days were 3.5° C. and 5.0° C. respectively. The temperature in the water-jacket was kept at 4.5° C. and 5.5° C. respectively. In experiment No. 10 the period used was 20 minutes instead of one hour and the number of cc. of $Ba(OH)_2$ neutralized shows a correspondingly lower figure.

The Biometer

Plate 3 represents a modification of the Biometer described by Tashiro in 1917. As originally designed by Tashiro the apparatus was equipped to accommodate only very small animals

or pieces of tissue. The present modification is the result of an attempt to use the same principles in the determination of the CO_2 output from larger animals such as toads, etc. The general principles of the biometer have been explained by Tashiro in his book "A Chemical Sign of Life," but in describing the present modification I shall summarize briefly the use of the whole apparatus.

The construction of the respiratory chamber has been described elsewhere. A few slight changes make it usable for the Biometer as well as the titration apparatus. To summarize again the use of the chamber in its application to the present apparatus: CO_2 - free air under pressure is admitted thru the tube Q, passes thru the coil R R where it is cooled or warmed to the temperature of the surrounding water jacket and thence passes thru the tube U into the Biometer. This adjustment of the temperature of the pure air is really not very necessary for the present apparatus but by using this type of respiratory chamber, the one chamber may be made to serve either for the Biometer or for the titration apparatus. Also the arrangement here shown is advantageous for use with a smaller respiratory chamber to be described later.

The CO_2 - free air passes thru the three-way stopcocks 1, 2, and 3, and thru the tube V into the respiratory chamber L L, thence out thru the tube T. This is for the purpose of thoroughly rinsing the chamber with pure air between each two readings. After the current has flowed thru the chamber 10 or 15 minutes,

stopcock 2 is turned 90 degrees so that the current flows into chamber E snd the screw-cock 5 is closed. The mercury burette G is lowered until the level of the mercury in E is lowered past the three-way stopcock 4. Stopcock 4 is now turned so that the air current flowing into chamber E flows out thru X, and the current is allowed to flow for 5 or 10 minutes, thus thoroughly rinsing chamber E with pure air. Then stopcock 4 is closed and the three-way stopcock 5 is turned in such a way that the current flows down thru tube C and out at tube Y. After about 5 minutes the stopcock 5 is closed and the CO_2 - free air supply is shut off. The apparatus is now thoroughly rinsed out and ready for use. Screw-cock 6 is now closed and screw-cock 7 is opened. Mercury burette K is raised so as to fill J with mercury to the level of stopcock 3. The mercury funnel N is now raised, thus allowing the mercury to fill tube V, pass beyond the three-way stopcock 3 to the level Z. (In this procedure the air above the rising mercury column may be allowed to escape thru the tube C, stopcock 5 and tube Y.) Screw-cock 7 is now closed tightly and N is lowered, leaving V full of mercury. Next the three-way stopcock 4 is turned in such a way as to leave a free passage from H to E and the mercury burette G is raised enough to fill the chamber E with mercury to the level of line B. Then stopcock 5 is turned so as to shut off any opening out thru the tube Y. Next quickly remove the cover from the respiratory chamber and place the cage P P containing the animal in position and replace the cover. The animal is now left in the cage for thirty

minutes or any other period of time and then a sample of air is removed and analyzed. The method of analysis is as follows:

With stopcock 2 turned obliquely (i.e. entirely closed) and stopcock 3 turned so as to connect I with V, screw-cock 6 is opened and the mercury contained in tubes V and I is allowed to flow downward thru 6 and thence into the respiratory chamber. The vacuum at first produced in tube I is later replaced by air which bubbles past the falling mercury. Thus tubes V and I are filled with air from the respiratory chamber, i.e. the air we wish to analyze. Now close screw-cock 6 tightly and open screw-cock 7 and raising the mercury funnel N if necessary so as to fill the lower part of tube V with mercury, thus compressing the air contained therein. Next turn three-way stopcock 3 in such a way that tubes I and V and burette J are all in communication. Mercury burette N is now slowly raised and K is simultaneously lowered so that the air held in V under pressure is forced over and into J. When V is completely filled with mercury up to stopcock 3, turn stopcock 3 so as to break the connection between I and J. Now adjust the mercury burette K so that the height of the mercury in K shall be the same as in J. This adjusts the pressure of the air in J to that of the outside atmosphere. The sample of air drawn from the respiratory chamber is now contained in burette J under atmospheric pressure, and ready to be tested.

At this point we should briefly review the general principles of the Biometer as described by Tashiro - The air to be analyzed is introduced into the chamber E. Then a half-

saturated solution of $Ba(OH)_2$ is allowed to flow down thru tube A, stopcock 5 and tube C and just enough of it is allowed to flow out on top of the capillary tube at D to form a hemi-spherical drop at D, and this is allowed to stand. If there is any CO_2 in the air in chamber E it will unite with the $Ba(OH)_2$ drop to form an insoluble precipitate of Ba_2CO_3 which may be detected with the aid of a hand lens. It has been determined that the least amount of CO_2 which will produce a precipitate on the drop of $Ba(OH)_2$ in ten minutes is 1.0×10^{-7} grams, and according to Tashiro this amount of CO_2 will produce a precipitate no matter how large a space the gas is occupying with the air. Accordingly if we introduce known quantities of our air sample into the chamber until a precipitate is formed, this amount of air contains $1.0 \times 10.^{-7}$ grams of CO_2. The method for introducing known quantities of the sample into the chamber E is as follows: The air is now contained in the upper part of J and the mercury in chamber E is at the level of line B. Turn stopcock 4 so as to allow a free passage from H to chamber E. Now carefully turn stopcock 5 so as to allow $Ba(OH)_2$ to flow up thru C, at the same lowering mercury burette G. When the $Ba(OH)_2$ solution is within one inch of D, close stopcock 5 tightly. Now turn stopcock 2 so as to allow a free passage from tube I into chamber E. (If the pressures in chamber E and burette J have been properly adjusted, no change should take place in the heights of the two mercury columns, when this connection between chambers is made). Now read and record the height of the mercury column in the burette

J. Slowly lower burette G and simultaneously raise burette K until the mercury column in J has risen a certain number of cc. This number represents the number of cc. of air introduced into chamber E. Now close stopcock 2 and very carefully open stopcock 5 until a drop of $Ba(OH)_2$ appears on the end of the tube at D. Close 5 tightly and wait 10 minutes, for the appearance of the precipitate in the drop. If at the end of this period no precipitate of $Ba(OH)_2$ is discernible with a hand lens, the same process may be repeated, raising burette K and lowering burette G, thus introducing a few more cc. of air into the chamber E. This process is continued until sufficient CO_2 is introduced with the air to produce a precipitate of Ba_2CO_3 on standing 10 minutes. Then the number of cc. of air introduced contains 1.0×10^{-7} grams of CO_2. Knowing the capacity of the respiratory chamber and the number of cc. of air in it which contain this amount of CO_2 it is a simple matter to calculate the number of grams of CO_2 given off by the animal in the experimental period. This is usually calculated in terms of grams of CO_2 per kilogram weight of animal per hour.

Use of the Small Respiratory Chamber

Figure 9 shows a small respiratory chamber for use either with the Biometer or with the titration apparatus. Such a chamber might be useful for studying the CO_2 output of insects

or other small forms which would not require the large chamber shown in Figure 6.

To use it in connection with the Biometer, the procedure is as follows: Place the animal in the small screen cage C', which is fastened to the glass stopper B', and insert the stopper in place. With stopcock 8 open, lower the mercury burette F' until the height of the mercury column in the chamber A' is lowered into the tube D'. With stopcock 3 properly turned raise mercury burette K until the level of mercury in J is at Z. Close stopcock 8 and leave the apparatus for the desired period of time. Then remove a sample of air for analysis as follows: Turn stopcock 1 so as to connect chamber A' with the tube U'. Turn stopcock 2 so as to connect tube U' with I. Thus we obtain a continuous passage from chamber A' to burette J. Now by simultaneously raising burette F' and lowering burette K a portion of the air in chamber A' is drawn over into burette J. Turn stopcock 2 so as to sever connection with chamber A' and the air sample in J is ready for analysis as previously described.

To use chamber A' with the titration apparatus, turn stopcock 1 so as to connect chamber A' with tube U and connect stopcock 8 by rubber tubing with tube G (Plate 2, figure 5). CO_2-free air is now allowed to pass thru the temperature control coil R R (Plate 3) up thru tube U and stopcock 1 into chamber A', and out thru stopcock 8; thence into the titration apparatus, where the method of analysis is the same as that described previously in connection with the large chamber.

VI. Summary

1. Contact with moist soil seems to be absolutely essential to the health of the toad, because:
   - A. Animals which fell into the green-house window pits and were not removed were usually found dead when surrounded by dry leaves and soil, alive if surrounded by damp leaves and soil.
   - B. Animals kept in the green-house died if the leaves and soil around them became dry.
   - C. Of nineteen toads kept in cages in the laboratory without water, eleven dried up and died, having lost 46.7% of their weight on an average. The other eight dried up and became quite stiff and apparently lifeless, losing 36.8% of their weight, but were revived later and restored to a normal healthy condition by drenching them with water.
   - D. In all these cases death appeared to be brought about or at least accompanied by a gradual drying up and stiffening of the animal's body.
   - E. Toads kept in flower-pots full of mud or in aquaria with a few inches of water in the bottom remained healthy and normal.
2. Some sort of absorptive force of the skin seems to be an important factor in preserving the normal water content of the toad's body because:

A. Toads which have partially dried out rapidly take in water when placed in a vessel of it.

B. Toads kept in mud usually remain plump and healthy, being somewhat swollen by the amount of water imbibed.

C. Preliminary experiments on the absorptive force of the toad's skin seem to indicate that:

1. In the live skin osmotic action goes on more readily from the outside of the skin toward the inside.
2. When the same solution is placed on both sides of the skin a current frequently flows thru the skin from the outside surface of the skin toward the inside indicating that some sort of absorptive force is operating apart from the usual osmotic action of an ordinary non-living membrane.
3. This absorptive force is stronger in skin taken from the back of the animal than in that taken from the ventral surface.
4. The absorptive force varies with the solution used and with the water content of the animal at the time when the skin was removed.

Acknowledgments

My thanks are due to Dr. V. E. Shelford for the original suggestion of the problem dealt with in this paper and to Dr. Shelford and others of the Department of Zoology of the University of Illinois for many helpful hints and suggestions during the course of the work.

Graphs

In the following graphs, figures on the ordinates indicate the number of millimeters by which the column of liquid in one tube of the osmometer exceeded that in the other tube. Figures on the abscissas represent time in hours.

Graph 1. Showing the effect of ordinary osmosis thru the fresh toad's skin. Skin taken from the ventral side of a toad. 5% cane sugar solution on one side of the skin; distilled water on the other. Curve X shows the effect when the sugar solution was on the inner side of the skin; curve Y when the sugar solution was on the outer side.

Graph 2. Ordinary osmosis. Same as graph 1 except that in place of the sugar solution some water expelled from the bladder of a toad was used as the liquid of greater density.

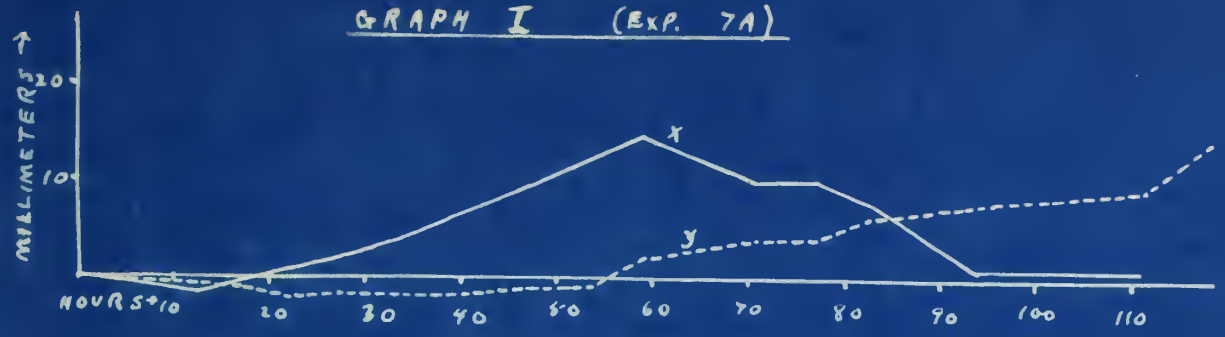
Graph 3. Showing effect when the same solution is on both sides of the skin. Different effects with different solutions. Skin removed from the ventral surface of the animal.

Graph 4. Same as graph 3 except that the skin was removed from the back of the animal.

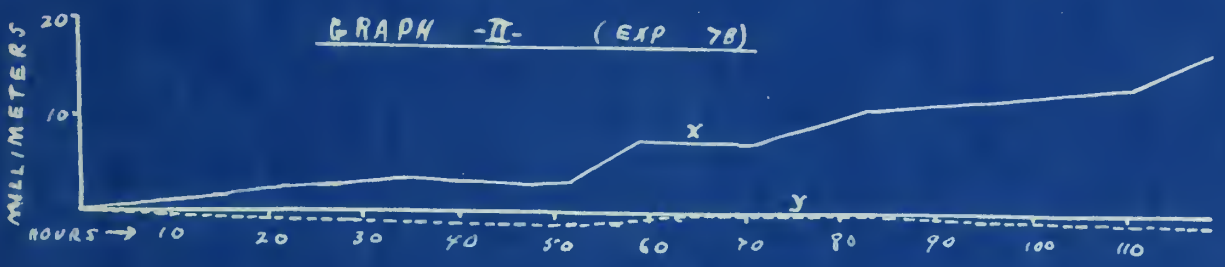
Graphs 5, 6, & 7. Use of the same solution on both sides of the skin. Skins taken from three different animals which varied in the relative amounts of water contained in their bodies.

Graphs 8 & 9. Use of the same solution on both sides of the skin. Specimen partially dried before removing the skin. (Loss in weight - 31 gms.). Comparison of skin taken from the ventral surface with that from the back of the animal.

GRAPH I (EXP. 7A)



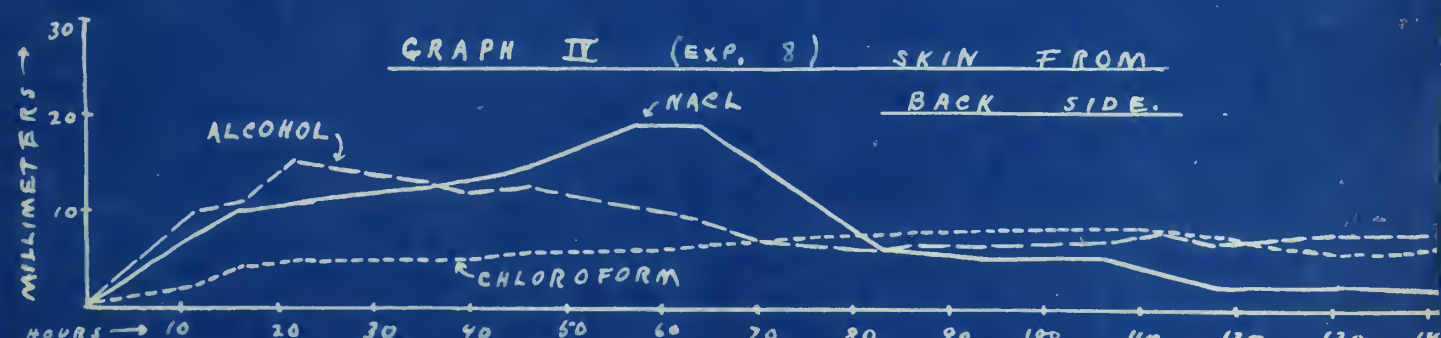
GRAPH -II- (EXP 7B)

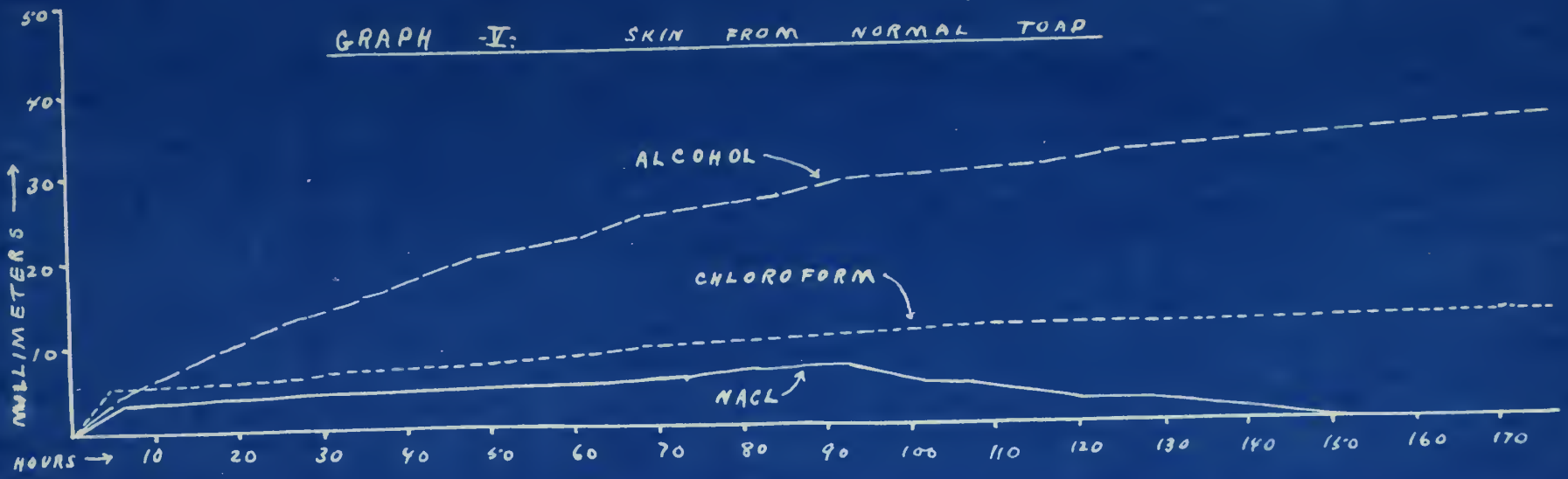


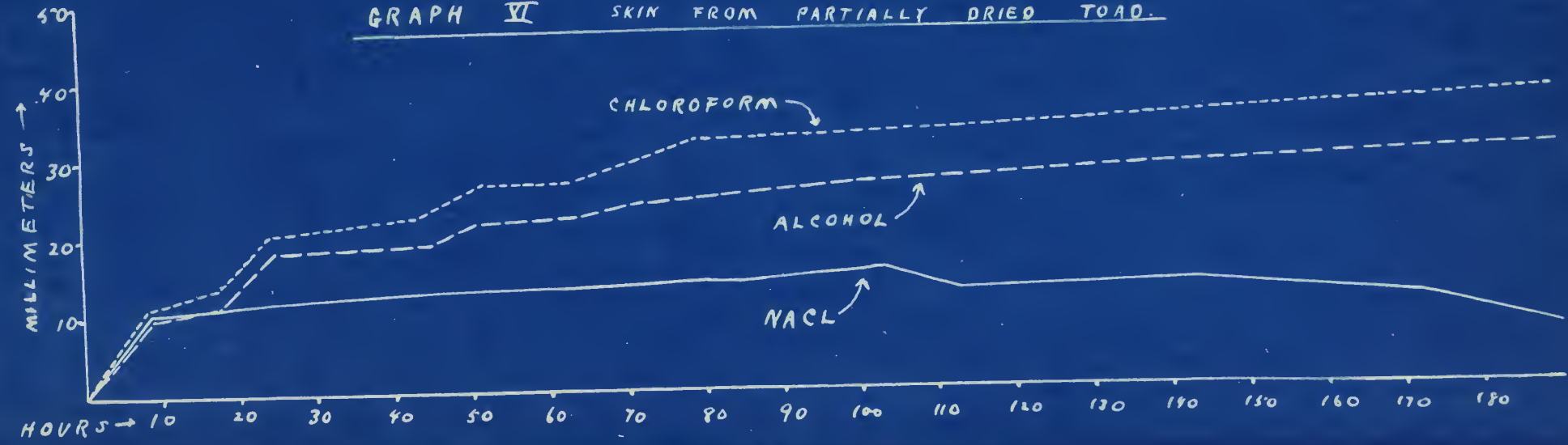
GRAPH III (EXP. 8)

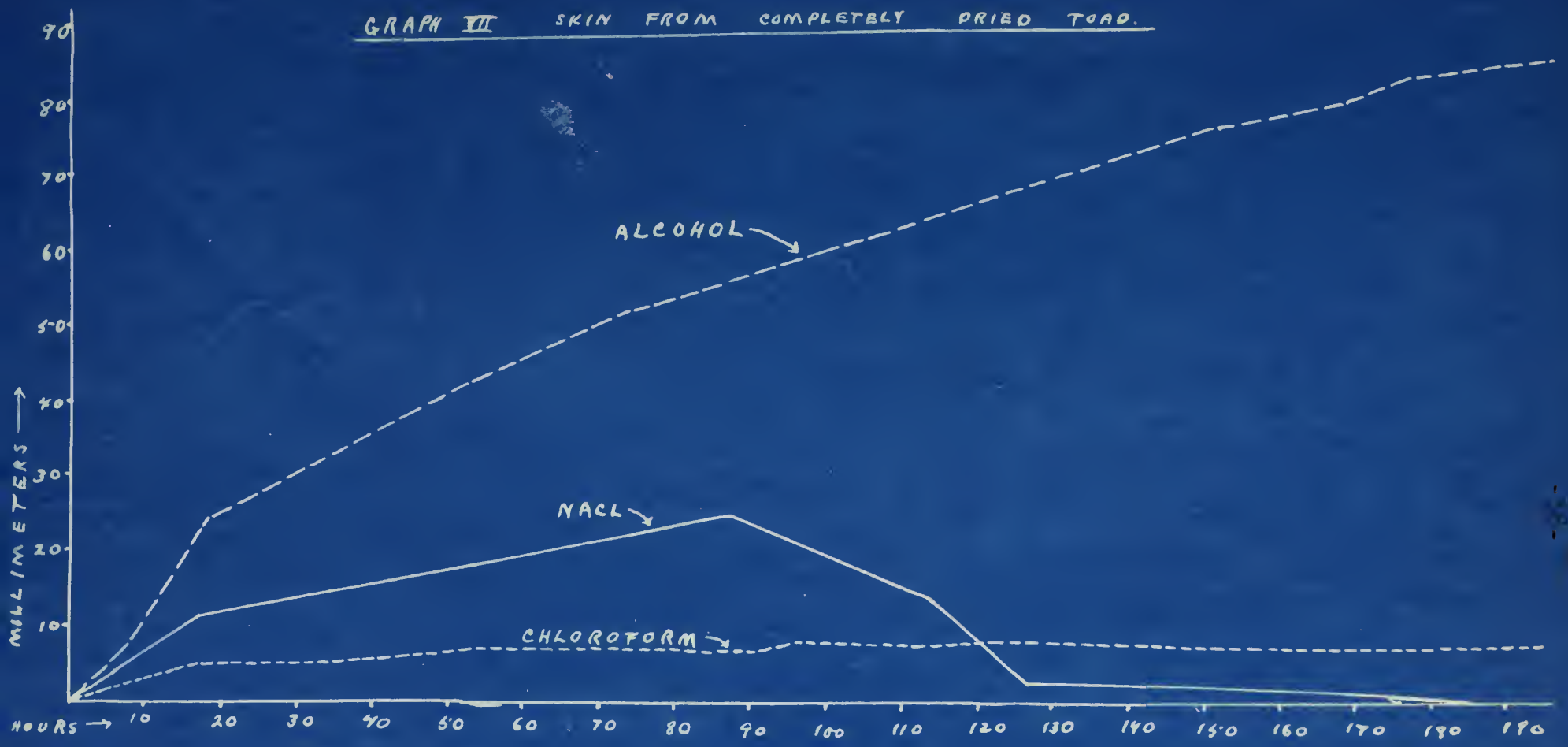
SKIN FROM BELLY SIDE.

GRAPH II (EXP. 8) SKIN FROM BACK SIDE.

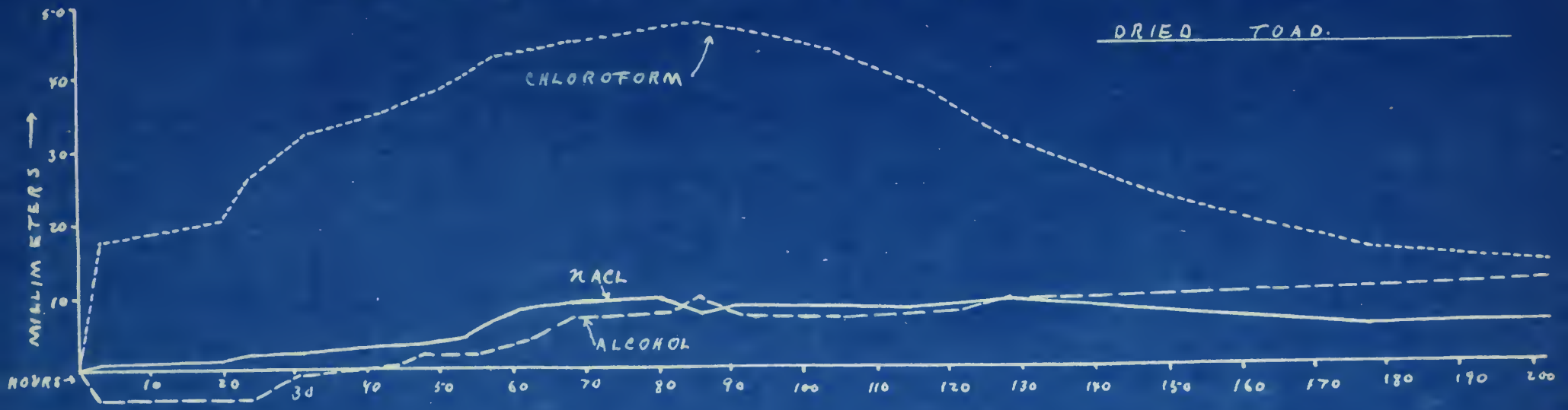



GRAPH V: SKIN FROM NORMAL TOAD
MILLIMETERS →
50
40
30
20
10
ALCOHOL
CHLOROFORM
NACL
HOURS → 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170

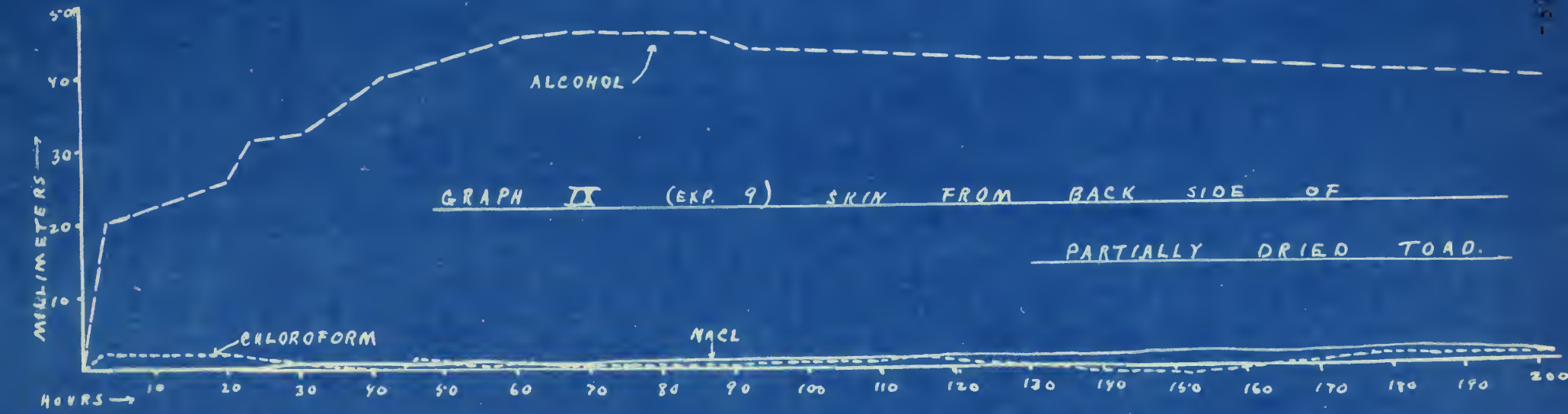

GRAPH VI SKIN FROM PARTIALLY DRIED TOAD.
MILLIMETERS →
50
40
30
20
10
CHLOROFORM
ALCOHOL
NACL
HOURS → 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180


GRAPH III SKIN FROM COMPLETELY DRIED TOAD.
MILLIMETERS
HOURS
ALCOHOL
NACL
CHLOROFORM
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

GRAPH VIII (EXP. 9) SKIN FROM BELLY SIDE OF PARTIALLY DRIED TOAD.



GRAPH IX (EXP. 9) SKIN FROM BACK SIDE OF PARTIALLY DRIED TOAD.



Plates

Plate 1. Apparatus and equipment. Fig. 1. Feeding cage; Fig. 2. The flower pot type of inclosure; Fig. 3. The osmometer; Fig. 4. Apparatus for obtaining CO_2 - free air.

Plate 2. Apparatus for determining CO_2 by titration; Fig. 5. The titration apparatus; Fig. 6. The respiratory chamber; Fig. 7. The manometer for regulating the pressure in the respiratory chamber.

Plate 3. The biometer. Fig. 8. The apparatus as modified to accommodate animals as large as toads; Fig. 9. The small respiratory chamber for insects, etc.

PLATE 1.

FIG 1.

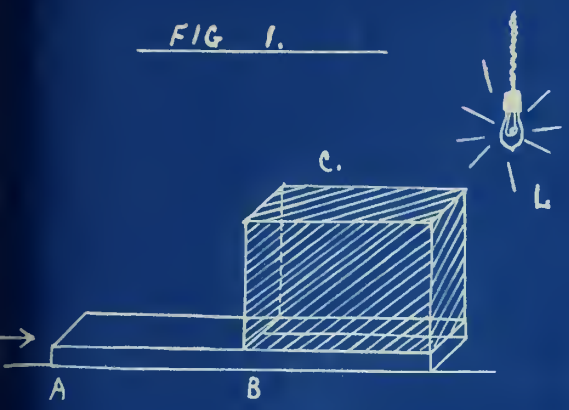


FIG 2.

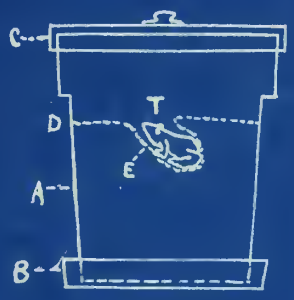


FIG 3.

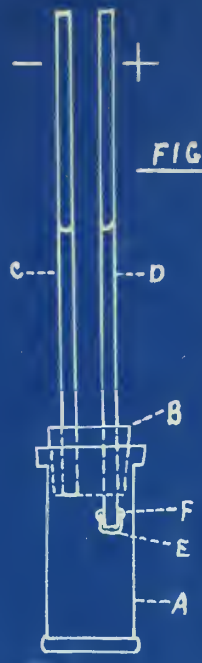


FIG 4.

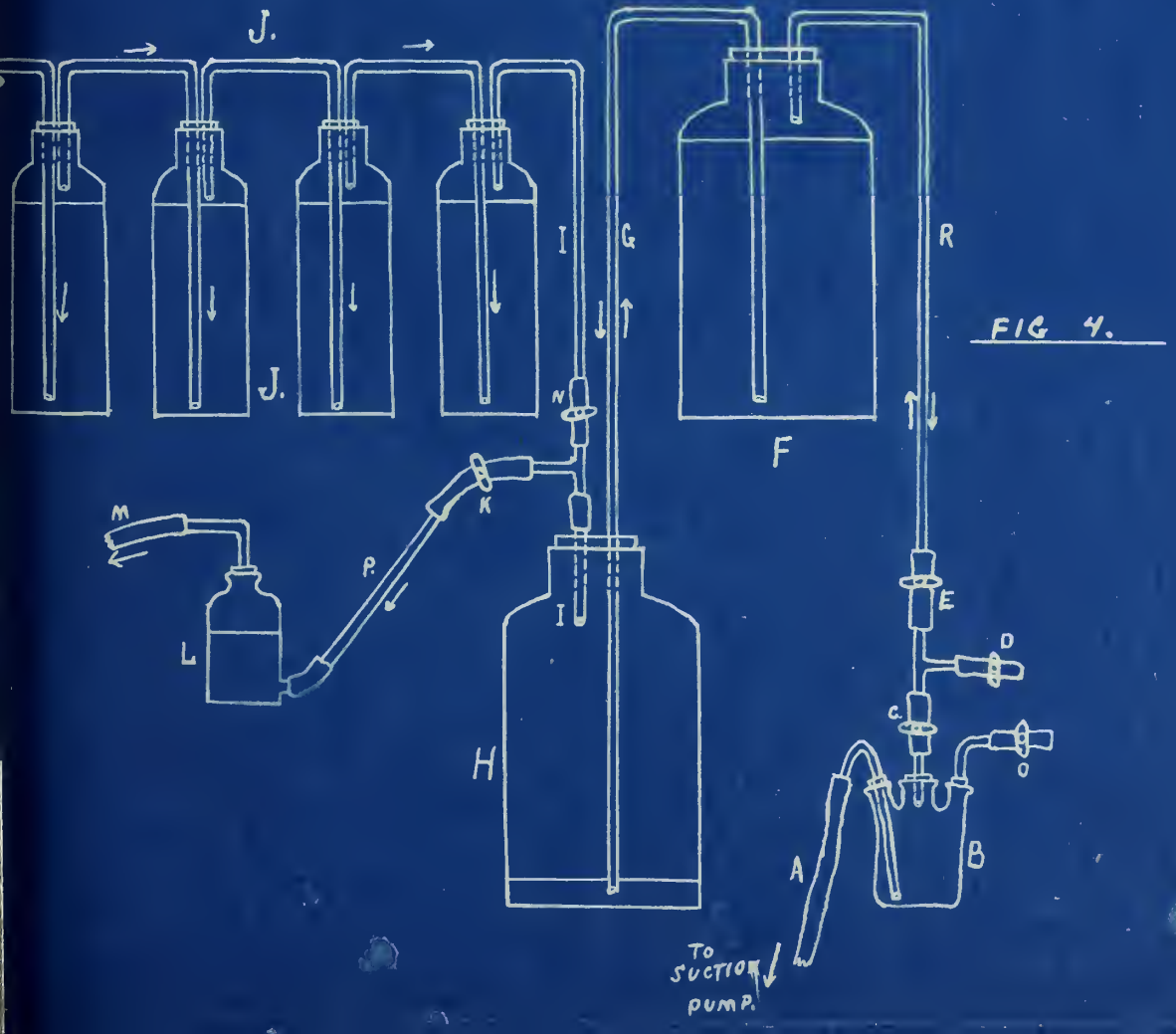
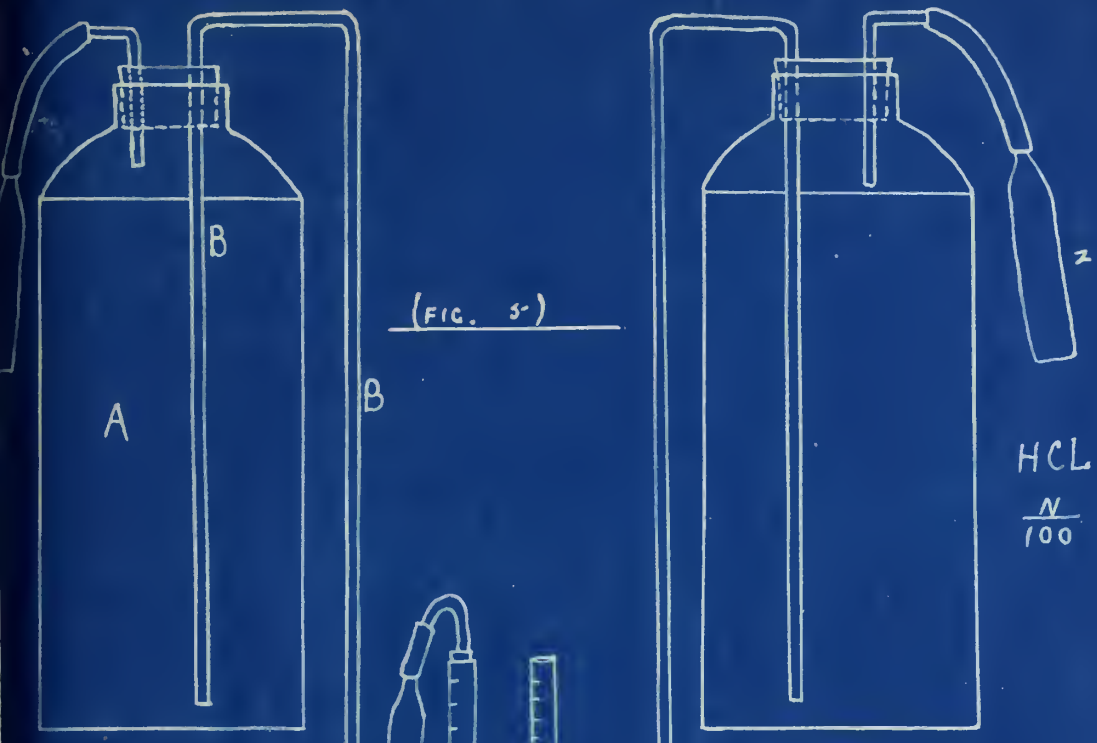


FIG 4.

PLATE 2.



(FIG. 5-)

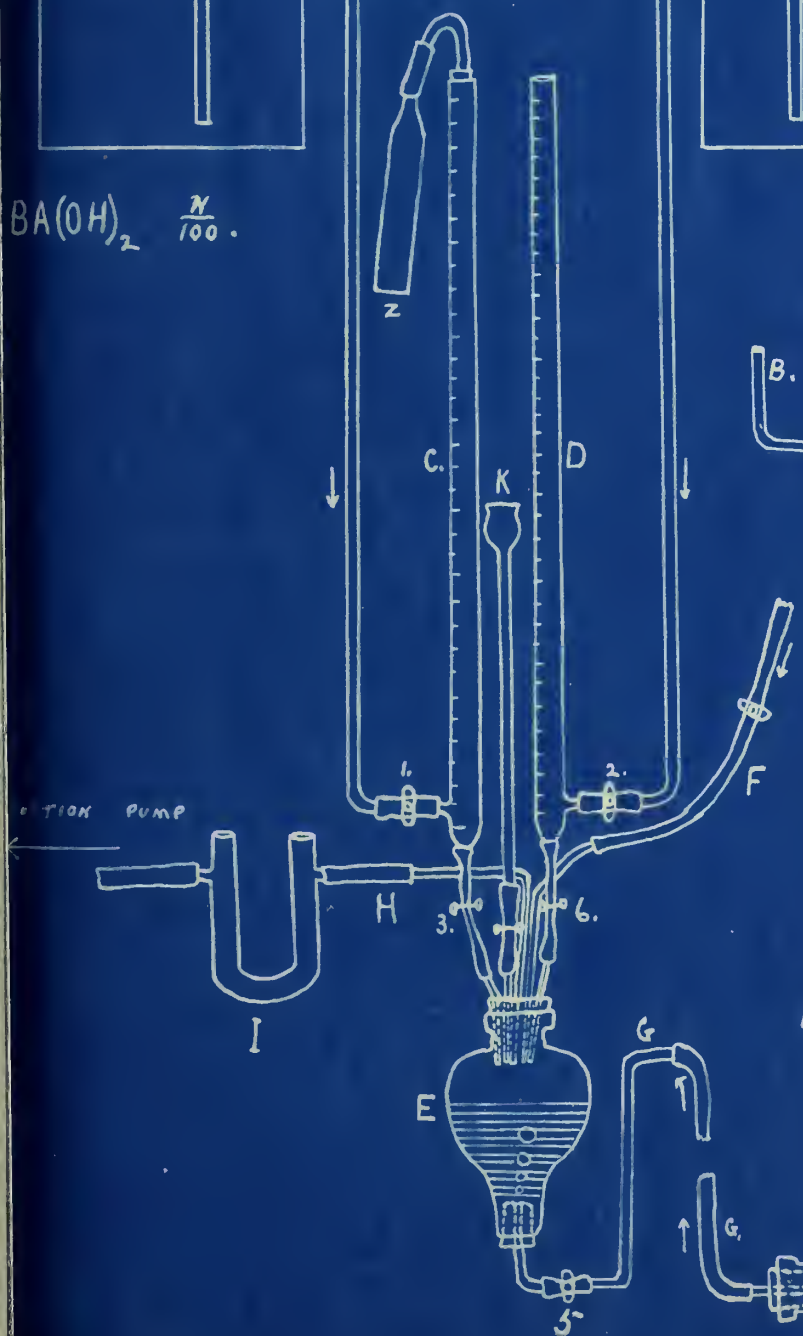


FIG 7.

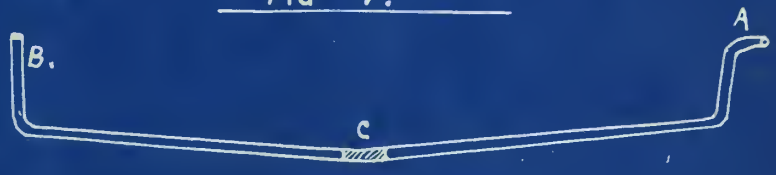


FIGURE 6.

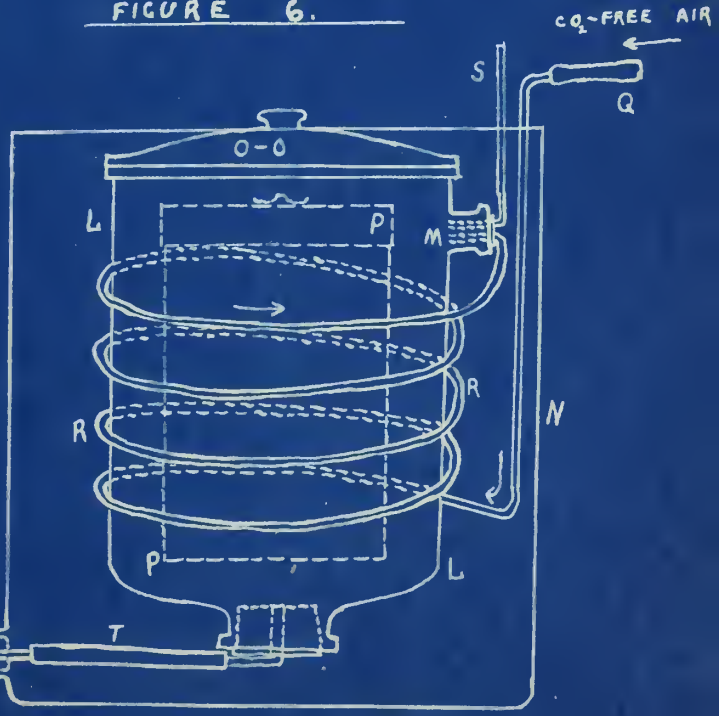


PLATE 3.

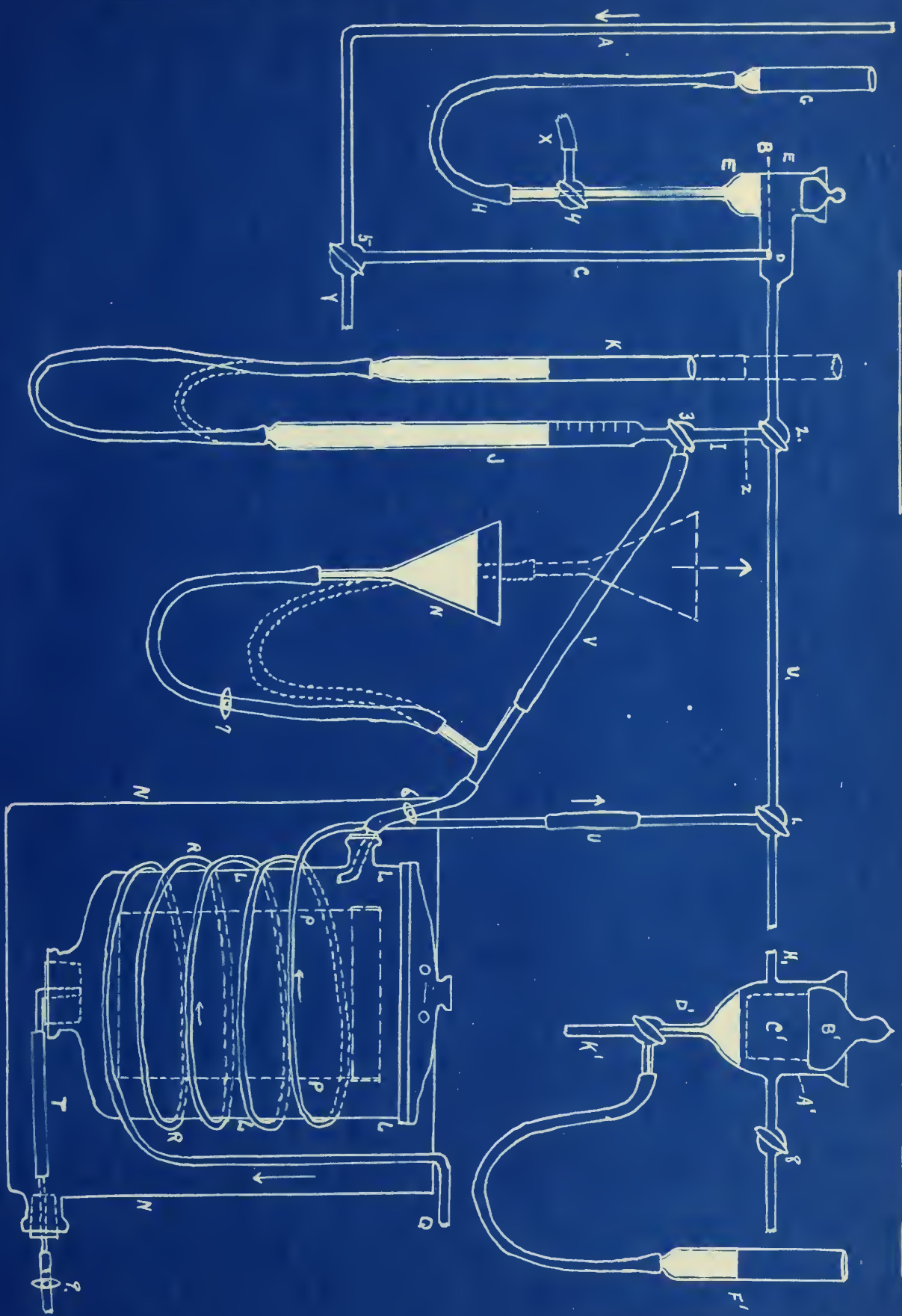


FIGURE 8

FIGURE 9

TABLE I Determination of the water content before and after drying, after being replaced in water, etc.

	Max.	Min.	Aver.
Wt. in gms. at beginning of experiment	99.0	52.5	71.4
Wt. of survivors after 52 hrs. drying	60.2	41.0	49.9
Loss in gms. after 52 hrs.	39.5	24.3	30.7
% of weight lost	58.0	27.0	42.0
Wt. of survivors after drenching	133.2	80.9	98.5
Gms. of water taken in after drenching	73.0	39.9	48.9
% of weight lost by 8 survivors	42.0	27.0	36.8
% of weight lost by 11 dead animals	58.0	37.0	46.5
Weight of 11 dead animals	62.4	21.8	36.1

TABLE II Showing the method of recording readings on the osmometer. Height of the two columns of liquid were measured in millimeters at intervals. Columns in the table marked indicate tubes covered by skin, those marked indicate open tubes. Differences between the two columns are recorded under "diff."

Date (1)	Time (2)	Hrs. (3)	.6 % NaCl (4) +	(5) −	(6) diff.	5 % alcohol (7) +	(8) −	(9) diff.	weak chloroform (10) +	(11) −	(12) diff.
5/6	10:30 P.M.		68.0	68.0	00	83.5	83.5	00	71.5	71.5	00
7	9:30 A.M.	11.0	68.0	60.0	8.0	81.0	71.0	10.0	72.5	7:.0	2.5
	2:30 P.M.	5.0	68.0	58.0	10.0	76.0	65.0	11.0	72.5	68.5	4.0
	8:30	6.0	68.0	57.0	11.0	73.0	58.0	15.0	73.0	68.0	5.0
8	9:30 A.M.	13.0	68.0	56.0	12.0	65.0	52.0	13.0	73.0	68.0	5.0
	1:30 P.M.	4.0	68.0	55.0	13.0	62.0	50.0	12.0	73.0	68.0	5.0
	8:30	7.0	68.5	53.5	15.0	58.0	45.0	13.0	73.0	67.0	6.0
9	8:00 A.M.	11.5	69.0	50.5	19.0	57.0	41.5	10.5	73.0	67.0	6.0
	2:30 P.M.	6.5	68.5	49.5	19.0	48.0	38.5	9.5	73.0	66.5	6.5
	8:30	6.0	66.0	51.0	15.0	44.0	37.0	7.0	73.0	66.0	7.0
10	8:30 A.M.	12.0	61.0	54.0	7.0	40.0	34.0	6.0	73.0	66.0	7.0
	12:30 P.M.	4.0	60.0	53.5	6.5	39.0	32.0	7.0	73.0	65.0	8.0
	7:30	7.0	58.5	53.0	5.5	37.0	30.0	7.0	73.0	65.0	8.0
11	8:30 A.M.	13.0	57.0	52.5	5.5	34.5	27.0	7.5	72.5	64.5	8.0
	2:30 P.M.	6.0	56.0	52.0	4.0	33.0	25.0	8.0	72.0	64.0	8.0
	8:00	5.5	55.5	53.0	2.5	32.0	25.0	7.0	72.0	65.0	7.0
12	8:30 A.M.	12.5	55.5	53.0	2.5	30.5	23.0	7.5	72.0	66.0	6.0
	8:30 P.M.	12.0	54.0	52.0	2.0	28.0	20.5	8.5	71.5	65.0	6.5
13	8:30 A.M.	12.0	53.0	51.0	2.0	27.0	18.0	9.0	71.0	65.0	6.0
Increase in mm. over original height			1	0		0	0		2.5	0	
Maximum difference					19			15			6.5

TABLE III Results of experiments on
how this force varies wit
solutions used,etc. In al
placed on both sides of t

Date	Exp. no.	Sex	Condition of animal	Source of skin	Ar in
1921					
May	2		partially dried	belly	ou
	3		normal	"	
	4		"	"	i
	5		"	"	ou
	6		"	"	i
	8		"	back	ou
	9A		partially dried	belly	ou
	9B		partially dried	back	
1920 May	IV		normal	A belly B back C "	
	III		partially dried	A belly B back C "	
	II		completely dried (dead)	A belly B back C "	

TABLE III Results of experiments on the absorptive force of the toad's skin, showing how this force varies with the condition of the animal, source of the skin, solutions used, etc. In all experiments here shown the same solution was placed on both sides of the skin.

Date	Exp. no.	Sex	Condition of animal	Source of skin	Arrangement of skin in osmometer	Condition of skin when used	Millimeters of difference between the two columns of liquid. A max. effect in .6 % NaCl	B max. effect in 5% alcohol	C max. effect in weak chloroform
1921 May	2		partially dried	belly	outside down	fresh	0	1.5	1.5
	3		normal	"	" "	"	0	1.0	22.5
						in water			
	4		"	"	inside down	10 min.	1.0	0	1.0
	5		"	"	outside down	fresh	4.0	1.0	7.0
	6		"	"	inside down	"	0	1.0	1.0
	8		"	back	outside down	"	19.0	15.0	6.5
	9A		partially dried	belly	outside down	A fresh B NaCl 3hrs C " "	3.5	4.0	35.0
	9B		partially dried	back	" "	A fresh B NaCl 3hrs C " "	1.0	40.0	2.5
1920 May	IV		normal	A belly B back C "	" "	fresh	7.0	36.0	12.5
	III		partially dried	A belly B back C "	" "	"	14.5	35.0	37.0
	II		completely dried (dead)	A belly B back C "	" "	"	24.0	83.0	8.0

TABLE IV Showing the difference in the osmotic force exerted by skin from a normal toad as contrasted with that from one partially dried; also between skin from the belly side and that from the back side of the animal. Figures represent maximum differences in millimeters between the two columns of liquid in the osmometers used.

Condition of animal	Solutions used	Skin from belly	Skin from back
	Experiment no.	5	8
Normal	NaCl	4.0	19.0
	Alcohol	1.0	15.0
	Chloroform	7.0	6.5
	Experiment no.	9A	9B
Partially dried	NaCl	3.5	1.0
	Alcohol	4.0	40.0
	Chloroform	35.0	2.5

TABLE V Showing actual increases in the height of the liquid columns in various experiments.

Solution	Experiment numbers						
	3	4	5	6	8	9A	9B
.6 % NaCl	0	0	0	0	1.0	1.0	0
	0	0	0	1.0	0	0	0
5 % alcohol	2.5	0	0	0	0	1.0	0
	2.0	0	0	0	0	0	0
weak chloroform	2.0	0	1.0	0	2.5	6.5	1.5
	0	0	0	0	0	0	0

TABLE VI Carbon dioxide output of twelve toads as determined by the titration apparatus.

Date	No.	Condition of toad	Wt. of toad in gms.	Time in min.	cc N/100 $Ba(OH)_2$ neutralized	Mg. CO_2 per kilo per hour	CO_2 per kg. per hr. divided by body wt.
1920							
10/30	1	Active	73.2	65	11.89	32.69	.446
	2		50.0	60	6.66	29.30	.586
	3		48.0	60	10.55	48.35	1.007
	4	Female, sleepy but not hibernating	88.0	60	9.84	24.60	.279
	5	Active	68.0	60	9.63	29.53	.434
11/1	6	Female	118.0	60	11.38	21.21	.179
	7	Sleepy, not in hibernating position	39.0	60	9.43	53.19	1.363
5	8	Female	109.0	60	15.37	31.02	.284
	9	Sleepy, in hibernating position	41.0	60	9.02	49.40	1.180
	10		73.0	20	3.08	27.84	.381
13	11	Hibernating position moved when touched	40.0	60	8.71	47.90	1.197
14	12	Same, stiffer but still alive	40.	60	3.69	20.29	.507

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